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Conients

ORIGINAL ARTICLES

PUBLISHED PROCEDURES RECOMMENDED FOR TRIAL

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ERRATUM

After page 11, line 18, Technical Supplement to Volume 7, No. 3, May, 1937, to read:

(b) Add this solution (*a*) slowly to 200 cc. of 95 per cent ethyl alcohol [instead of 20 cc.].

SUBSCRIPTION PRICE $1.50 PER VOLUME

A SIMPLE METHOD FOR MAKING NON-METALLIC AND NON-FRAGILE OPEN MESH CUPS FOR TRANSFERRING TISSUE BLOCKS AND BASKETS FOR SUPPORTING THEM IN THE UPPER LEVELS OF DEHYDRATING SOLUTIONS*

I. A. NELSON

From the Pathological Laboratories of St. John's Hospital, Tulsa, Oklahoma, aided by the Mark Finston Research Fund

These cups and baskets were developed after unsatisfactory attempts to use wire screen cups, aluminum tea strainers, coffee percolators and perforated porcelain ware. Metals amalgated with the mercury which we used to seal our dehydrating jars. Porcelain was expensive, fragile and desirable sizes unobtainable.

The only materials needed for these cups and baskets are:

1. Curtain material of the so called "fillet net" weave. The cross fibers do not spread in this material. This makes it possible to knead the glue into the threads without fear of distorting the mesh. We have found the 16 point grade most generally useful.

2. Casein glue made by LePage or under the "Casco" trade name can be bought in almost any store. It is important to follow the instructions on the containers.

The cups can be made as follows:

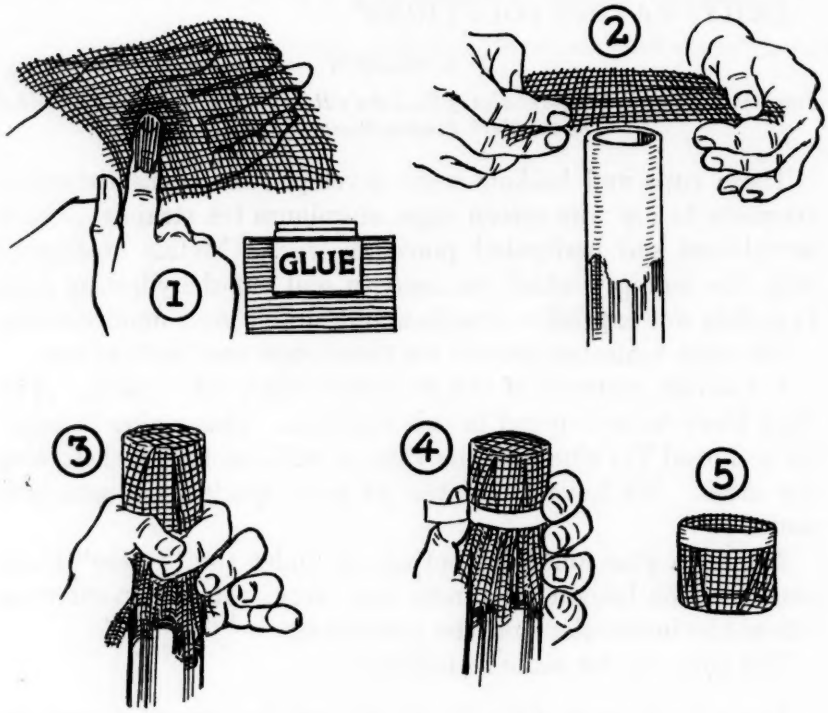
Take a 4 inch square of the fillet net and work the prepared glue into the threads. Knead increasing amounts of glue into the fabric until it begins to show some spaces filmed over when the square is stretched open. Mold this over the open end of a large test tube which has been coated with paraffin. We use the 1 inch Nessler tubes. Put some glue on a strip of good bond paper about ¼ inch wide and about 6 inches long. Wrap this around the fabric and tube about 1 inch below the open end of the tube. Set aside in a rack to

* Methods and Apparatus shown at the Scientific Exhibit Section of the Fifteenth Annual Meeting of the American Society of Clinical Pathologists, May 6–10, 1936, Kansas City, Missouri.

dry. Trim off the dangling corners by rotating the tube while a razor blade is held at the lower edge of the paper strip. Warm the tube to soften the paraffin and slip the molded cup off. The paper serves to reenforce the top of the cup and eliminates the presence of a ragged thready edge.

The baskets can be made in three different ways:

The fabric with glue may be pressed into the mouths of the paraffin coated containers, or they may be molded over wide vessels of the desired size in the same manner as the cups are made, or they may be made on special forms



made to suit the particular jars to be used. This method gets around the difficulty of finding molds of just the right size and hastens drying.

1. Impregnate a piece of dry heavy cardboard such as comes with X-ray films with hot paraffin. Cut out a hole in the center of the cardboard of exactly the same size inside diameter as that of the vessel to be used. We use 4 by 4 inch glass dressing jars. For this size we start with a piece of cardboard about 9 inches square.

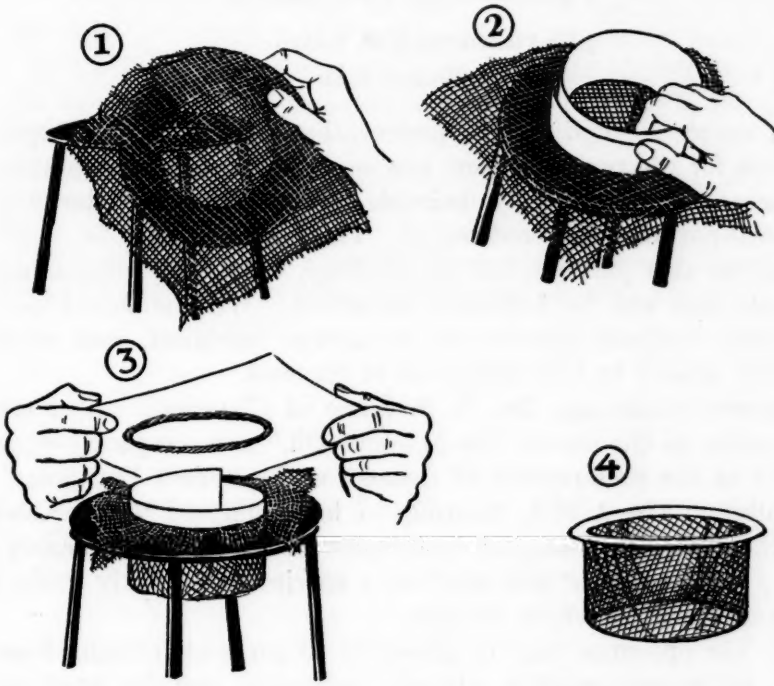
2. Impregnate a strip of heavy manilla paper about 18 inches long and about 3 inches wide with hot paraffin.

3. Take a piece of good quality bond paper and cut out a hole in this of the same inside diameter as in the vessel to be used.

4. Take a piece of fillet net about 9 inches square and impregnate with glue.

5. Spread the net over the heavy cardboard form which should now be placed on a ring stand, or large can, or wire basket.

6. Make a scroll of the paraffined manilla paper about 3½ inches in diameter and firmly push this down on the net so that it is forced down through the central opening to about 1¾ inches. Permit or force the manilla scroll to open



as much as it can within the central opening. Wrinkles and irregularities in the net can be smoothed out at this stage.

7. Now put an excess of glue on the fillet net, limiting it to about ¼ inch just around the central hole and next to the manilla paper scroll. Thread the bond paper down over the scroll so it rests firmly on the net. Apply pressure with an inverted jar, can, or some sand. This serves to make a reenforced shoulder on the basket.

8. When the glue is dry, remove the pressure, roll the scroll out of the center and separate the net from the paraffined cardboard. The bond paper should be

firmly attached to the net and should be trimmed down to nearly ⅛ of an inch. The resulting basket fits snugly in the 4 inch dressing jar and the reenforced edge rests on top to keep the basket from slipping down. The bottom of this basket is about 2½ inches above the bottom of the jar. The glass cover of the dressing jar should clear the reenforced edge without sticking.

TEMPORARY PRESERVATION OF TISSUES

(A Method Especially Adaptable to the Clinical-Pathological Conference)

FREDERICK H. LAMB

Davenport, Iowa

A simple method for the preservation of surgical or autopsy tissues for the period of from one week to two or three months, if necessary, is especially desirable in connection with hospital clinical-pathological conferences. Permanency may be sacrificed for this purpose, but morphology, color, and consistency should and can be faithfully preserved. With standard permanent methods consistency is always sacrificed, and color cannot always be fully preserved or restored.

Several years ago, Dr. B. S. Kline of Cleveland, called my attention to the use of "Ice Machine Oil," as a temporary expedient in the preservation of tissues for the "Fresh Pathology" Exhibit at the A.M.A. meeting. I have adapted this method to the clinical-pathological conferences in the following manner:

1. With water or salt solution, a specimen is quickly washed free of excess blood, or exudate.

2. The specimen may be placed in 10 per cent formalin from five to twenty minutes without distortion, and for practical purposes without color change. The thin film of protein fixation is especially desirable in warm weather. This immersion in formalin may be omitted altogether, if the specimen is to be used within ten days or two weeks.

3. Carefully blot the excess wash water or formalin, and allow the surface of the specimen to dry in the air from two to five minutes.

4. Attach an identifying label, immerse the specimen in

"Superla Oil,"* and store the container in a refrigerator, preferably about 5 to 8°C.

5. To display a specimen, remove it from the cold oil, allow the excess oil to drip away, and place the specimen in a clean white enamel pan of suitable size, preferably a separate pan for each specimen.

6. If care is taken to avoid excess water, blood, or exudate the same oil may be used for several sets of specimens.

7. Depending somewhat on their nature, specimens may be faithfully preserved in this manner for as long as six months.

If the foregoing method has been published elsewhere, I have not seen it, and I desire to acknowledge my thanks to Dr. Kline for the original suggestion. The method is so simple, inexpensive, and satisfactory that it deserves a trial by any one interested.

* We prefer to use the oil marketed under the name of "Superla White Rose" by the Standard Oil Co., retailing at approximately 50 cents per gallon.

GROWTH OF TUBERCLE BACILLI ON VARIOUS MEDIA WITH SPECIAL REFERENCE TO LEGUMES*

GEORGE B. LAWSON AND A. W. BENGTSON

Roanoke, Virginia

Because of widespread interest in culture methods for the recovery of tubercle bacilli from microscopically negative specimens, there have appeared in the literature on this subject numerous formulae of primary importance for dependable culture media especially suitable for the cultivation of this organism.

Since many of the media, in part or in whole, contain potato and egg, and because of the remarkable property exhibited by these agents of stimulating growth of tubercle bacilli, it occurred to us to initiate a new inquiry to determine whether or not the protein complex in legumes also would not manifest growth factors of a certain specificity for its development.

In a large number of experiments this proved to be true; the results of studies with more than ten thousand cultures, using twenty-two varieties of legumes and fifty-two strains of human tubercle bacilli, were significant and of especial interest in that certain legumes were found to possess a definite capacity for the proliferation of tubercle bacilli.

In this series of experiments, extending over a period of more than two years, (1935–36) quantitative and qualitative evidence of a uniform degree demonstrated growth factors of marked specificity in such legumes as the English split peas (dried), canned green peas, dried blackeyed peas, striped crease back beans, Kentucky wonder wax beans, white beans, October beans, lima beans and many others.

The striking affinity of tubercle bacilli for such legumes as English split peas, green peas, blackeyed peas and others showed a diminution, with decline of growth, on cow peas, soy beans and Michigan black beans, with finally the clover family and alfalfa group refusing repeatedly to yield growth (Table 1). It is difficult at this time to make a definite classification subdividing the legumes into groups as to growth factors, for repeated experi-

* Received for publication April 23, 1937.

mentation has proven that both the legume and its liquid are essential as nutritive material. Many combinations have been tried, yet classification and ultimate proportions are still indeterminate.

TABLE 1

VARIETIES OF LEGUMES AND CONTROLS USED

1. English split pea	Very good
2. Green pea	Very good
3. Blackeyed pea	Very good
4. Navy bean	Good
5. October bean	Good
6. Lima bean	Good
7. Striped crease back bean	Good
8. Kentucky Wonder wax bean	Good
9. Lima bean juice	Good
10. White potato (modified) (control)	Good
11. Soy bean	Fair
12. Cow pea	Fair
13. Michigan black bean	Fair
14. Green pea juice	Fair
15. Sweet potato	Negative
16. Yellow clover	Negative
17. Red clover	Negative
18. Alfalfa	Negative
19. Beet	Negative
20. Carrot	Negative
21. Indigo	Negative
Controls	
22. Petragnani	Very good
23. Miraglias	Very good
24. Corper's Potato Cylinder	Very good
25. Sweaney and Evanoff	Fair
26. Petragnani and green pea	Very good
27. Petragnani and blackeyed pea	Very good
28. Miraglias with English split pea (juice)	Very good
29. Miraglias with green pea (juice)	Very good

As some of the nutritive substances in many of the standard culture media for the cultivation of tubercle bacilli do not in themselves sustain growth, it occurred to us to fortify them with legumes; that is, to incorporate legumes and their juices in part as stimulating growth factors, partially replacing the usual com-

ponents such as milk and white of egg, which are the constituents of many media. A series of studies involving hundreds of quantitative tests with highly diluted positive sputa produced gratifying results. It should be borne in mind, however, that study and experimentation in this subject must be carried a great deal further before the exact proportion of legume to be incorporated into standard culture media can be determined precisely and with assurance; for it is generally known that heavy seedings are no indication of the efficiency of a medium. It is therefore evident that for practical purposes, before differentiating efficiency, a great deal of work must yet be done with legumes, using microscopically negative sputa, that is, from suspected cases containing comparatively few bacilli, critically discriminating for wide or constant divergency, with controls of the most efficient standards.

Our chief concern so far has been the testing of growth factors of legumes for the human type of tubercle bacilli, and critical observation of more than ten thousand cultures has unquestionably proven that the organism has selectivity for this food. It is deemed advisable, however, to caution against the promotion and wide use of these new media for microscopically negative specimens until further work has been done, for legumes and their extracts show signs of complexity in their response to the growth of tubercle bacilli.

The technique chosen for this work, using known positive sputa and pure legumes, is as follows:*

Sterilize in the hot air oven cotton-plugged 150 mm. x 18 mm. test tubes, 2-cc. pipettes, 50-cc. centrifuge tubes (corked), glass drawn 8-inch pipettes, several pieces of folded gauze, No. 7 cork stoppers with 3-mm. vent cut lengthwise and a few 10-cc. pipettes with extreme tip chipped off to permit free flow of medium.

If legumes are of the dried variety, soak about 200 grams in 700 cc. of water

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* The technic described is for the preparation of media containing legumes only, not for standard media modified by the addition of legumes. All the technical work has been done in the laboratory at Catawba Sanatorium. This study was shown as an exhibit at the Meeting of the Southern Medical Association in Baltimore, November 17th-20th, 1936.

for about 24 hours; then change the water and autoclave at 15 lbs. for about 25 minutes. Mix 30 cc. of the supernatant autoclaved bean juice with 180 cc. distilled water. Drain autoclaved legumes through coarse sieve for a few minutes, and mash part of this through fine sieve. Weigh out 105 grams of pureed beans and mix with above water and bean juice. Place in a 500 cc. flask and add 3 grams of granulated agar. Autoclave at 15 lbs. for 20 minutes; then add immediately 7 cc. glycerine and the appropriate dyes.[1] Do not add too much dye. Thoroughly mix the hot medium by whirling and set aside to cool to 50–60 degrees C. Judging temperature by hand is sufficiently accurate. With canned peas, lima beans, etc. soaking and first autoclaving are of course omitted.

An hour or so before tubing this medium, place a small block of ice in a tray or some suitable receptacle, with walls about one inch high. Place basket of empty sterile culture tubes on top of ice to cool. A bucket of cracked ice may be used instead. The tubes must be *thoroughly* cooled.

When the medium is at the proper temperature—that is, a little above solidification—mix and draw out about 13 to 14 cc.; quickly wipe off outside of pipette with inside of folded sterile gauze and transfer to culture tube in slanting position. One pipette and two pieces of gauze are sufficient for each batch of medium. Slant tubes without rotating, on the edge of the tray with the butt in ice water. This manipulation, though simple, is important to prevent contamination, and insures quick solidification and a homogenous medium with a clear front for colony observation. The medium should be used within forty-eight hours, for it dries readily.

In destroying secondary bacteria such as found in sputa, Corper's and Cohen's method[2] is used with slight modification. From 5 to 20 cc. of sputum is thoroughly homogenized with an electric shaker. With a sterile pipette draw out 2 cc. and carefully insert into the bottom of a sterile 50-cc. centrifuge tube, being careful not to touch the sides or mouth of the tube. Two cubic centimeters of 5 per cent oxalic acid is mixed with the sputum. Place pipette against centrifuge tube about one inch above sputum and with a whirling motion allow the acid to run down the side of tube into sputum. Now mix sputum and oxalic acid mixtures thoroughly by drawing up into pipette several times, avoiding bubbles as much as possible, and place in incubator for 45 to 60 minutes; *not longer*. Mix by whirling the sputum-acid mixture every 10 to 15 minutes, add approximately 40 cc. sterile saline, thoroughly whirl and centrifuge.

Pour off supernatant fluid, flame lip and mix sediment with sterile, glass-drawn pipette; draw up sediment with same pipette and transfer 2–3 drops on each culture tube. With microscopically negative sputa we inoculate 2–3 drops in each of 4 or 5 culture tubes. With known positive sputa one loop full, 4 mm. diameter, is sufficient for each culture. If sediment is thick, dilute with a few drops of sterile saline. Flame lip of culture tube and insert sterile cork with vent, placing small piece of cotton very loosely into vent.

Place culture tubes in incubator in slanting position for a few days, then place in upright position in tin cans having 8–10 holes in lid.

With known positive sputum, colony formation should appear macroscopically in 12–14 days.

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(2) Corper, H. J., and Cohn, M. L.: Jour. Lab. and Clin. Med., **18**: 515–520. 1933.

A FIVE AND ONE-HALF HOUR TECHNIC FOR PARAFFIN SECTIONS

MARTHA A. WHITE

Church Home and Infirmary, Baltimore

The following technic for paraffin sections has proved so satisfactory in our hands that it is offered for publication with every assurance that those who try it will not be disappointed.

Method:

1. Boil fresh tissue in 10 per cent formalin.
2. Place blocks in acetone for 1 hour.
3. Change to fresh acetone for 1 hour.
4. Benzine 10 minutes.
5. Chloroform and paraffin equal parts 20 minutes.
6. Paraffin 2 changes 1 hour each.
7. Cut, mount sections on slide and allow to dry in oven for 30 minutes.
8. Stain and mount.

The method is recommended especially for uterine curettings but is satisfactory for other tissues except extremely fatty ones.

A RAPID, EASY AND RELIABLE IRON HEMATOXYLIN STAIN FOR AMEBIC CYSTS IN FECES

W. S. THOMAS

Attempts to shorten the time of staining amebic cysts in feces by the time honored iron alum hematoxylin of Heidenhain have not met with complete success in my hands. Over ripening of the alcoholic solution of hematoxylin, acidity or some other reason has caused too often, brownish or muddy green preparations, furthermore, the irregularity in the action of iron alum as a decolorizing agent has caused some difficulty.

The uniformly beautiful nuclear stains obtained in sections by using Regaud's hematoxylin and decolorizing with picric acid-alcohol, as described by Masson,[1] suggested a trial on feces specimens containing cysts. The method was successful from the start and has continued so. The chromatin ring of the nucleus, the centrosome and chromatoid body stain as well as with any other stain I have seen. All stained elements are a deep blue instead of brown, black or green.

Solutions required:

1. Schaudinn's fluid with acetic acid:
   Saturated aqueous solution of mercuric chloride, 2 parts.
   Alcohol 95 per cent, 1 part.
   Glacial acetic acid sufficient to make 2 per cent.
2. Iron ammonium sulfate (ferric): 5 per cent aqueous solution.
3. Regaud's hematoxylin:
   Hematoxylin, 1 gram.
   Alcohol 95 per cent, 10 cc.
   Glycerin, 10 cc.
   Distilled water, 80 cc.
   Dissolve the hematoxylin in hot water, cool and add the alcohol and glycerin. It may be used immediately.
4. Picric acid-alcohol:
   Saturated alcoholic solution of picric acid, 2 parts.
   Alcohol 95 per cent, 1 part.

Method: Coplin jars containing the Schaudinn's fluid, the iron alum solution and the hematoxylin are heated in a water bath or oven to 45 to 50 degrees Centigrade.

1. Fix thin films on slides in heated Schaudinn's fluid for 5 minutes.
2. Rinse in 50 per cent alcohol.
3. Place in 70 per cent alcohol to which sufficient Lugol's solution has been added to give a rich brown color, 5 minutes.
4. Place in 95 per cent alcohol 5 minutes to remove excess iodine and to harden.
5. Hydrate by bringing through 70 and 50 per cent alcohol to water.
6. Place in heated iron alum solution for 5 minutes.
7. Rinse well in tap water.
8. Place in heated Regaud's hematoxylin for 5 minutes.
9. Dip for a moment in 95 per cent alcohol and transfer to the picric acid-alcohol for 10 to 20 minutes.
10. Wash in running tap water for 5 minutes. If permanent preparations are desired, wash for 15 minutes.
11. Dehydrate, clear in xylol, mount in balsam.

In publishing this method no claims for originality are made. Tuan[2] in 1930 advocated the use of a saturated aqueous solution of picric acid as a differentiating fluid in iron alum hematoxylin stain and Hunter[3] again in 1934, while Regaud's hematoxylin as a stain for protozoa is mentioned in 1929 by Wenrich.[4] The other steps described, except as to time and temperature, are those in general use.

The superiority of Regaud's hematoxylin over Heidenhain's lies only in the superior keeping qualities of the former. Heidenhain's is entirely satisfactory when fresh and even old, cloudy solutions have at times yielded satisfactory preparations when differentiated with the picric acid-alcohol mixture instead of iron alum.

SUMMARY

An easy, reliable iron alum hematoxylin stain for amebic cysts in feces is described which can be completed in about one hour.

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PUBLISHED PROCEDURES RECOMMENDED FOR TRIAL

BLOOD GROUPING AND COMPATIBILITY. A SIMPLE TECHNIC FOR DETERMINATION FROM SMALL AMOUNTS OF DEFIBRINATED WHOLE BLOOD

Paul Hoxworth and Azel Ames, J. A. M. A., **108:** 1234. 1937

"In municipal hospitals heavily burdened with traumatic surgery, the method employed for grouping and matching blood prior to transfusion must be chosen for speed as well as accuracy. While meeting modern requirements for safety, the technic will of necessity be designed primarily for efficiency."

The procedure the authors consider ideally suited to these needs is a combination of the Vincent open macroscopic method for grouping and the Coca compatibility test for direct matching. Each method has been slightly modified so that both determinations can be made with a single, small sample of defibrinated whole blood.

Method:

Grouping: 1. Approximately ten drops of blood are obtained by finger puncture and defibrinated in a Wassermann tube by whipping with a wooden applicator for five minutes.

2. Large drops of high titered test serums of anti-B and anti-A are placed on the left and right ends respectively of a glass slide.

3. Defibrinated blood is added to each drop of serum and thoroughly mixed with the aid of a platinum loop.

4. The slide is tilted back and forth over a well lighted white background and observed macroscopically for clumping.

Agglutination is striking if serum of high titer is used.

When no agglutination is seen after one minute slide is placed under a petri dish with wet blotting paper and observed fifteen minutes later as a precaution against weak agglutinogens.

Matching:

1. Obtain blood from recipient and defibrinate as above.

2. A 1:1 or 50 per cent suspension of the recipients blood is prepared in the stem of a white cell counting pipet by drawing blood to the 0.5 mark and then filling to the 1.0 mark with physiologic salt solution.

The diluted blood is deposited on the left end of a glass slide and mixed by means of a jet of air blown from the pipet.

3. An identically prepared suspension of donor's defibrinated blood is placed on right end of the same slide and one-fifth of this drop (two divisions of the pipet) is transferred to the drop of diluted recipient's blood and stirred with a platinum loop or glass rod and placed under a petri dish with wet blotting paper.

This 1:5 ratio is based on the assumption that 500 cc. is to be transfused to a patient whose blood volume has been depleted 50 per cent or approximately 2500 cc. It provides a wide margin of safety in transfusions from a universal donor, as dangerously potent agglutinins will not be completely absorbed in this concentration without manifest agglutination.

4. After fifteen minutes the mixture is again agitated and observed microscopically under low power for agglutination.

Advantages claimed by authors:

1. Both grouping and compatibility may be determined with ten drops of defibrinated whole blood obtained by finger puncture.

2. Venipuncture and separation of cells and serum are unnecessary.

3. The veins of patients requiring repeated intravenous therapy may be conserved.

4. Provided standardized, high titered test serums are employed the blood group is usually obvious in less than one minute.

5. Compatibility may be determined with a wide margin of safety in fifteen minutes.

6. Time and material consumed in making cover slip preparations are saved.

7. "Universal" donors with agglutinin titer dangerous for A or B recipients can be detected by the 1:5 mixture of donor's and recipient's blood.

8. Low titered A donors may be selected for "universal" recipients.

9. The total elapsed time from the arrival of unknown donors to the final reading is reduced to thirty minutes.

DIRECT MICROSCOPIC EXAMINATION OF THE SKIN. A METHOD FOR THE DETERMINATION OF THE PRESENCE OF FUNGI

J. H. Swartz, Arch. Dermat. & Syph. **33:** 291. 1936

The use of clearing agents, such as sodium or potassium hydroxide, xylene, chloral hydrate, and glycerine, while permitting the microscopic examination of scales, has led, in many instances, to a positive diagnosis of fungus infection,

because of the presence of artefacts interpreted as fungi. The chief artefacts which simulate fungi are crystals, fat globules and the "mosaic fungus."

In searching for a stain which would select and differentiate the hyphae and spores from the various artefacts and surrounding epidermal cells the following procedure was evolved:

Solutions required:

1. Potassium Hydroxide, 5 per cent aqueous solution.
2. Lactophenol of Amann:
   Lactic acid, 1 cc.
   Phenol crystals, 1 gram.
   Glycerin, 2 cc.
   Distilled water, 1 cc.

To this may be added 0.5 per cent cotton blue (C4B Poirrier).

Method:

1. Clear scales in 5 per cent Potassium Hydroxide.
2. Wash in water 2 to 3 minutes.
3. Gently heat scales in lactophenol-cotton blue mixture.
4. Press cover glass on preparation and examine.

If permanent preparations are desired the stained scale may be mounted in the following mixture:

Distilled water, 50 cc.
Chloral hydrate, 50 grams.
Glycerin, 20 cc.
Acacia, 30 grams.
or the excess of lactophenol-cotton blue mixture may be removed and the cover slip sealed with Noyer's cement.

(Editor's note): The formula for Noyer's cement, as given by Langeron, is as follows:

Lanolin (anhydrous), 20 grams.
Colophonium, 80 grams.
Heat gently and mix thoroughly.

A SIMPLIFIED PEROXYDASE REACTION

ALBRECHT GRAF DOUGLAS-S, Chemiker Zeitung, February 6, 1937, No. 11, p. 130

The author found that the simplified procedure for carrying out the peroxydose reaction, as described by Hirschfeld (Klin. Woch., 14: 1437, 1935), frequently yielded overstained preparations, he attempted, therefore, to modify Graham's reaction and claims that his method will enable even unskilled

workers to obtain satisfactory results and moreover that it is possible to carry out fixation, peroxydase reaction and contrast staining in a single operation in 3 minutes.

Stock Solutions:

1. Dioxan saturated with benzidine (use free base!). The concentrated solution is diluted with ten volumes of dioxan.
2. Add 0.1 cc. 3 per cent H_2O_2 to 10 cc. of a 0.5 per cent aqueous solution of safranine.
3. Dioxan.

All solutions are quite stable.

Method:

1. Cover air dried blood films with Solution 1, 20 to 30 seconds.
2. Add equal number of drops of Solution 2, 2 to 3 minutes.
3. Pour off and wash with several drops of Solution 3.
4. Dry in air.

CONTRAST STAIN FOR THE RAPID IDENTIFICATION OF TRICHOMONAS VAGINALIS

J. R. MILLER, J. A. M. A., **106:** 616. 1936

A drop of 0.1 per cent safranin used as a diluent for the pus to be examined stains leucocytes, whereas the trichomonas remains unstained and conspicuous as a clear object against a slightly pink background. The dye in this concentration does not interfere with the motility of the organisms, indeed, it appears to stimulate it. Under the low power it is often possible, more quickly, to pick out area where the organisms are numerous, so that identification with the high power objective can be quickly effected.